

## **Methods of Rapid Kinetics**

To follow the kinetics of a chemical reaction, one needs to monitor the concentration of each molecular species (reactants and products) as a function of time. When the reaction is sufficiently slow, one can sample the reaction mixture at various times. When the reaction is a touch faster, but still relatively slow, it may be necessary to quench the reaction before analyzing the reaction mixture in terms of the reactant and product concentrations. When the reaction is faster still, one needs to follow the molecular species *in situ* as a function of time. This is usually accomplished by spectrophotometry if one or more of the molecular species exhibits a spectrum in some convenient region of the electromagnetic spectrum, e.g. when the molecular species is colored. In this experiment, one exploits Beer's Law or the Beer-Lambert Law:

$$A_{\lambda} = \log_{10} \frac{I_0(\lambda)}{I(\lambda)} = \varepsilon(\lambda) C l$$

where  $A_{\lambda}$   $\equiv$  absorbance at wavelength  $\lambda$

$C$   $\equiv$  concentration of absorbers

$l$   $\equiv$  pathlength of absorption cell

$I_0(\lambda)$   $\equiv$  incident light intensity at wavelength  $\lambda$

$I(\lambda)$   $\equiv$  transmitted light intensity at wavelength  $\lambda$

$\varepsilon(\lambda)$   $\equiv$  extinction coefficient at  $\lambda$

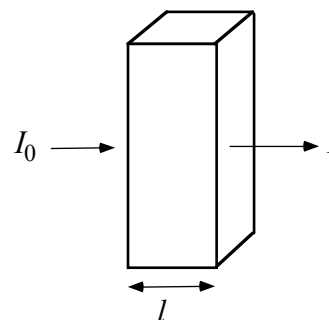


Figure 8-1

One can monitor the absorbance ( $A$ ) at one wavelength, or several wavelengths using a diode array spectrophotometer so that the concentration of several molecular species can be monitored simultaneously as a function of time.

Note that, according to the Beer-Lambert Law

$$A_{\lambda}(t) \propto C(t)$$

so that the time course of the molecular species monitored is provided by  $A_{\lambda}(t)$ .

Clearly the simple experiment works only when the reaction is slow compared to the mixing time of the reactants in the cuvet.

### **What Does One Do if the Reaction Is Faster than the Mixing Time?**

#### **(1) Stopped-flow Rapid Mixing**

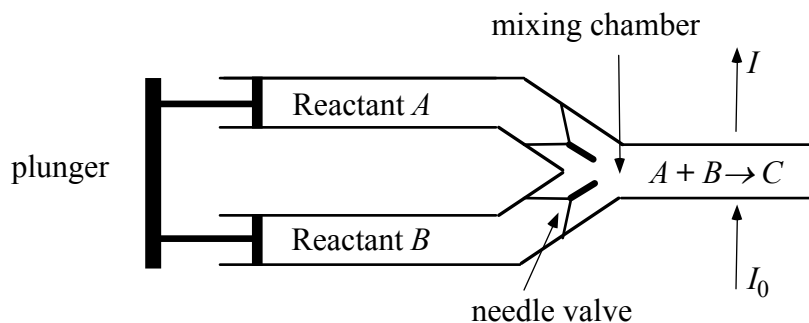


Figure 8-2

Mixing time is 1– 5 msec. Sample volume requirements are typically a few cc, so good for biochemical kinetics, where one is often limited by availability of enzyme and even substrate.

#### **(2) Continuous Flow Reactor (if one is not sample limited)**

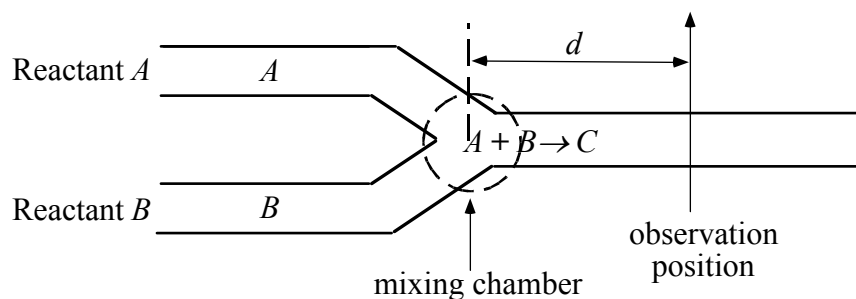


Figure 8-3

Time delay determined by “ $d$ ” and flow rates ~ a few msec.

#### **(3) Flow-flash**

This is a variant of the continuous flow reactor, except that the reaction is initiated by a laser pulse. Dioxygen chemistry of cytochrome *c* oxidase was studied at room temperature by flow-flash spectroscopy. CO is flashed off by laser pulse in a few nsec.

O<sub>2</sub> binds to the enzyme in less than 1 μsec (10<sup>-6</sup> sec). You can monitor dioxygen chemistry over the time window of 1 μsec → 10 msec depending on the size of the reaction zone monitored.

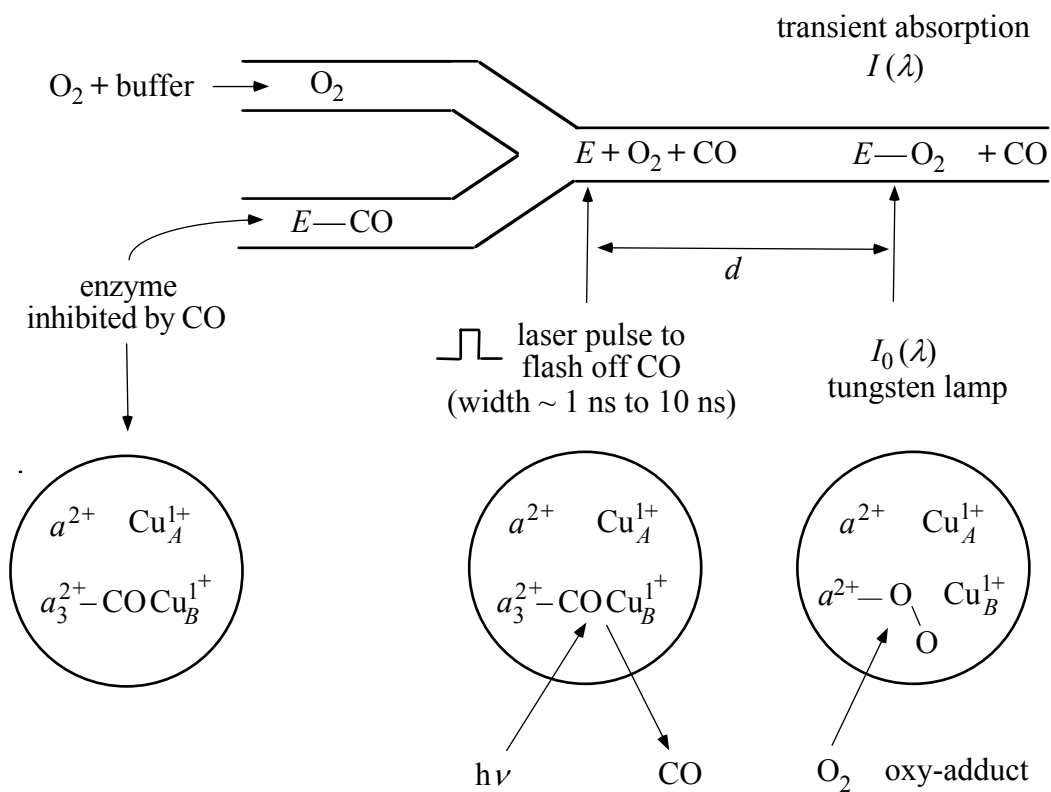


Figure 8-4

**(4) Transient Absorption**

In this procedure, a biochemical event is triggered by light or laser pulse, then the process is followed by time-resolved spectrophotometry. Electron input from cytochrome *c* to cytochrome *c* oxidase can be studied in this way.

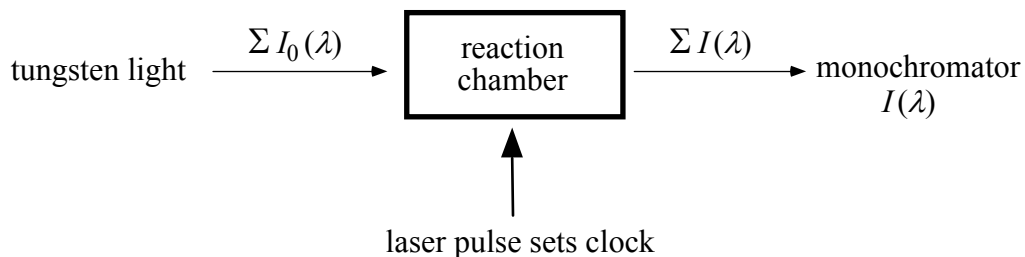
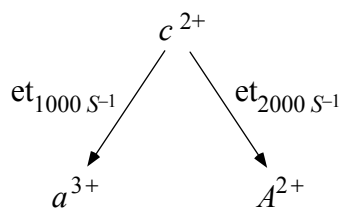
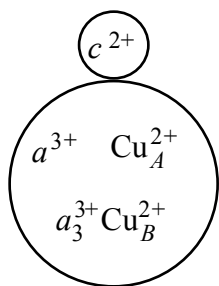
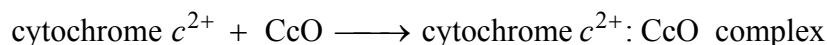
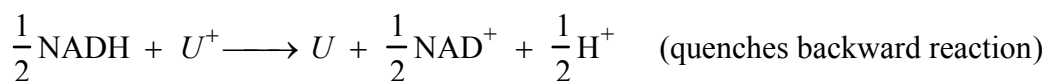
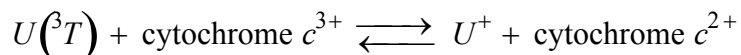
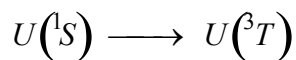


Figure 8-5

Recently, Chan's group developed the following technique to study electron input into cytochrome *c* oxidase.



- Monitor reoxidation of  $c^{2+}$  by absorption at 550 nm.
- Monitor reduction of  $a^{3+}$  by absorption change at 605 nm.
- Can monitor reduction of  $A^{2+}$  by absorption at 830 nm.

**(5) Perturbed Equilibrium Experiment (Relaxation Method)****Principle**

- (a) Shift or perturb equilibrium with a chemical impulse or physical impulse (T-jump, pressure jump).
- (b) Follow the approach of the system toward a new equilibrium by some time-resolved method.

Electron transfer or electron equilibration between  $a$  and  $\text{Cu}_A$  in  $\text{CcO}$  was studied this way.

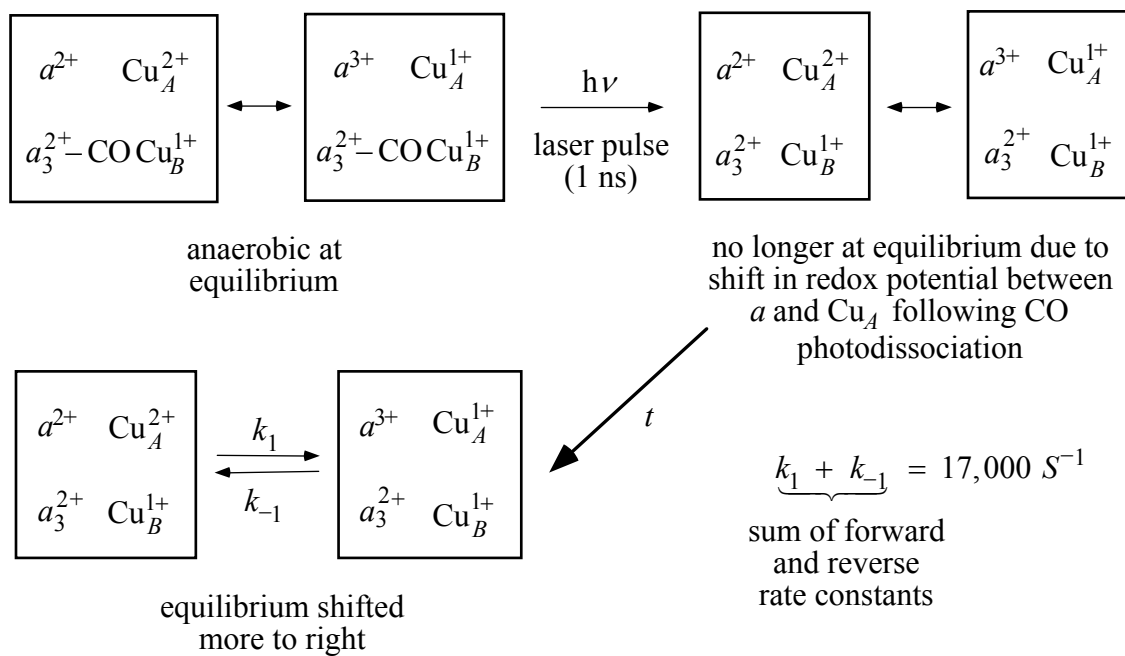
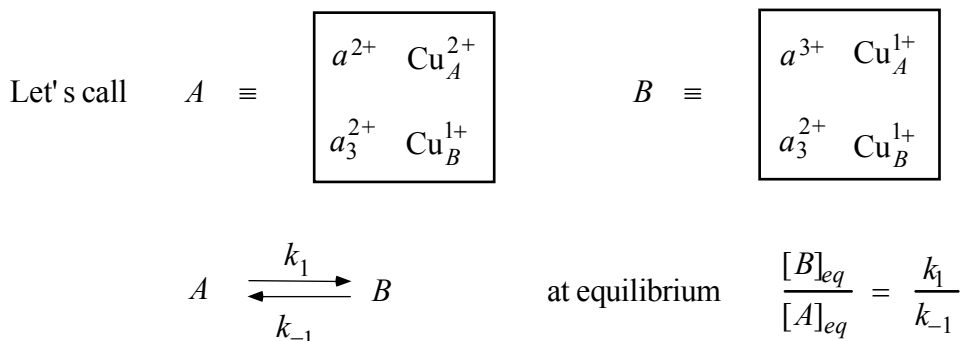


Figure 8-6

Let's examine this process in some detail.



Immediately following the laser flash, the system is not at equilibrium.

The approach toward equilibrium is governed by the following rate equations:

$$\frac{d[A]}{dt} = -k_1[A] + k_{-1}[B]$$

$$\frac{d[B]}{dt} = k_1[A] - k_{-1}[B]$$

Since  $[A] + [B] = C_0$

we may write  $\frac{d[A]}{dt} = -(k_1 + k_{-1})[A] + k_{-1}C_0$

Now  $[A]_{eq} + [B]_{eq} = C_0$  also

But  $[B]_{eq} = \frac{k_1[A]_{eq}}{k_{-1}}$

so  $[A]_{eq} + \frac{k_1[A]_{eq}}{k_{-1}} = C_0$

or  $(k_{-1} + k_1)[A]_{eq} = k_{-1}C_0$

Substituting into the rate equation

$$\begin{aligned} \frac{d[A]}{dt} &= -(k_1 + k_{-1})[A] + (k_{-1} + k_1)[A]_{eq} \\ &= -(k_1 + k_{-1})([A] - [A]_{eq}) \end{aligned}$$

or integrating

$$\left( [A(t)] - [A]_{eq} \right) = \left( [A(0)] - [A]_{eq} \right) e^{-(k_1 + k_{-1})t}$$

$$\text{or } \underset{\substack{\uparrow \\ \text{absorbance}}}{\Delta A_\lambda(t)} = \Delta A_\lambda(0) e^{-(k_1 + k_{-1})t}$$

In our study,  $\Delta A_\lambda(t)$  was monitored at 605 nm (*a* absorption) and 830 nm ( $\text{Cu}_A$  absorption).

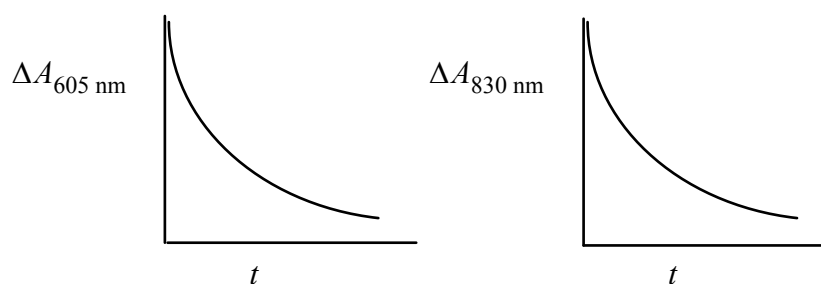


Figure 8-7

$$k_1 + k_{-1} \cong 17,000 \text{ S}^{-1}$$

### (6) Temperature Jump

This is a perturbed equilibrium experiment where the equilibrium of the system is shifted by a sudden change in temperature. Relaxation of the system toward a new equilibrium at the new temperature allows one to measure the relaxation times. It is generally assumed that  $\Delta T$  is sufficiently small that the  $k$ 's measured pertained to the system at the original equilibrium.

$$\Delta T \approx 5\text{--}10^\circ \text{ C}$$

Temperature jump is achieved by

- (a) Discharge of an electric capacitor through the solution.
- (b) Use of a rapid laser pulse.

**(7) Pressure Jump**

This is a perturbed equilibrium experiment where the equilibrium of the system is shifted by a sudden change in pressure.

Recall  $\left(\frac{\partial \ln K}{\partial P}\right)_T = \frac{\Delta V^0}{RT}$  so require  $\Delta V^0 \neq 0$

Pressure jump is achieved by suddenly rupturing a restraining diaphragm to release pressurization of the system.

$$\Delta P = 100 \text{ — } 1000 \text{ atm}$$

**(8) Equilibrium Methods**

This method is based on examining the rapid fluctuations of the system — analysis of noise.

- (a) Concentration fluctuations — detected as fluctuations in the absorption or fluorescence of molecular species.
- (b) Concentration fluctuations and density fluctuations reflected in refractive index variations that lead to variations in scattering of light from laser beams.
- (c) Pressure fluctuations, producing noise in a sensitive acoustic detector.
- (d) Lifetime broadening of energy levels leading to Heisenberg uncertainty broadening of spectral lines:  $\Delta \nu \Delta t \approx 1$ .



**More on Relaxation Times**

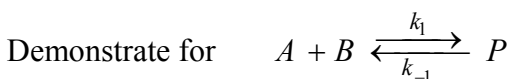
For reactions involving single steps, one can always write

$$-\frac{d(\Delta[P])}{dt} = \frac{\Delta[P]}{\tau} \quad \text{where} \quad [P] = [P]_{eq} + \Delta[P]$$

$\equiv$  product concentration  
 $\tau \equiv$  relaxation time

so that  $\Delta[P]_t = \Delta[P]_0 e^{-\frac{t}{\tau}}$

Mechanism	(Relaxation Time) <sup>-1</sup>
$A \xrightleftharpoons[k_{-1}]{k_1} B$	$\tau^{-1} = k_1 + k_{-1}$
$A + B \xrightleftharpoons[k_{-1}]{k_1} P$	$\tau^{-1} = k_{-1} + k_1 \cdot ([A]_{eq} + [B]_{eq})$
$A + B + C \xrightleftharpoons[k_{-1}]{k_1} P$	$\tau^{-1} = k_{-1} + k_1 \cdot ([A]_{eq}[B]_{eq} + [B]_{eq}[C]_{eq} + [A]_{eq}[C]_{eq})$
$A + B \xrightleftharpoons[k_{-1}]{k_1} P + Q$	$\tau^{-1} = k_1 \cdot ([A]_{eq} + [B]_{eq}) + k_{-1} \cdot ([P]_{eq} + [Q]_{eq})$
$2A \xrightleftharpoons[k_{-1}]{k_1} A_2$	$\tau^{-1} = 4k_1[A]_{eq} + k_{-1}$



Rate expression:  $\frac{d[P]}{dt} = k_1[A][B] - k_{-1}[P]$

at equilibrium:  $\frac{k_1}{k_{-1}} = \frac{[P]_{eq}}{[A]_{eq}[B]_{eq}}$

Apply a jump at  $t = 0$  (duration of impulse is typically  $10^{-6}$  S, but it can be as short as  $10^{-8}$  S. This defines an initial departure from equilibrium.

Write instantaneous time-dependent concentrations as follows (as system approaches new equilibrium created by jump).

$$[P] = [P]_{eq} + \Delta[P]$$

$$[A] = [A]_{eq} + \Delta[A] = [A]_{eq} - \Delta[P]$$

$$[B] = [B]_{eq} + \Delta[B] = [B]_{eq} - \Delta[P]$$

since  $\Delta[P] = -\Delta[A]$

$$= -\Delta[B] \quad \text{for this example}$$

### Boundary Conditions

$$t = 0 \quad \Delta[P] = \Delta[P]_0$$

$$t = \infty \quad \Delta[P] = 0$$

### Substituting into Rate Expression

$$\begin{aligned} \frac{d[P]}{dt} &= \frac{d([P]_{eq} + \Delta[P])}{dt} = \frac{d(\Delta[P])}{dt} = k_1[A][B] - k_{-1}[P] \\ &= k_1([A]_{eq} - \Delta[P])([B]_{eq} - \Delta[P]) - k_{-1}([P]_{eq} + \Delta[P]) \\ &= \underbrace{k_1[A]_{eq}[B]_{eq} - k_{-1}[P]_{eq}}_{= 0} - k_1 \left\{ ([A]_{eq}\Delta[P]) + [B]_{eq}\Delta[P] - \underbrace{(\Delta[P])^2}_{\text{ignore}} \right\} - k_{-1}\Delta[P] \\ &= - \left\{ k_1 \cdot ([A]_{eq} + [B]_{eq}) + k_{-1} \right\} \Delta[P] \end{aligned}$$

This is OK for small displacements from equilibrium.

Hence we obtain

$$-\frac{d(\Delta[P])}{dt} = \frac{\Delta[P]}{\tau}$$

where  $\tau = \left\{ k_{-1} + k_1([A]_{eq} + [B]_{eq}) \right\}^{-1}$

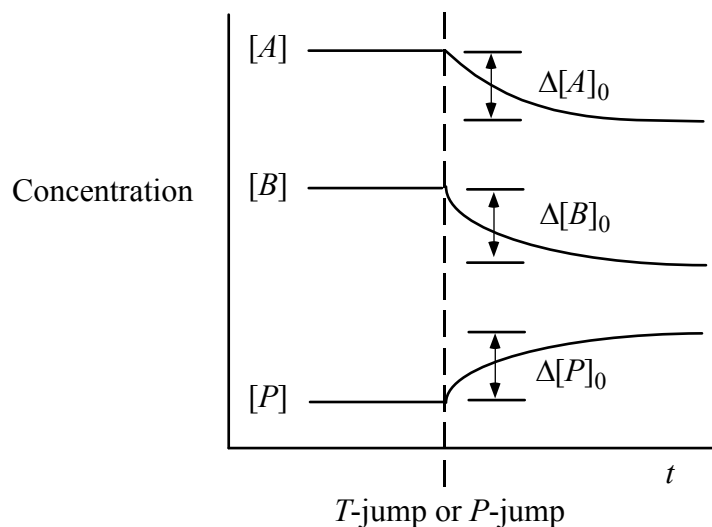
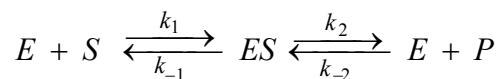


Figure 8-8

### Enzyme Catalyzed Reactions

The enzyme catalyzed reactions are more complicated; there are at least two steps and there is at least one intermediate state that exists (enzyme-substrate complex) and participates in the relaxation process.



At equilibrium or under steady state conditions, the concentrations are governed by

$$\frac{k_1}{k_{-1}}, \quad \frac{k_2}{k_{-2}}$$

and a perturbation, such as a temperature jump will influence these ratios. So the relaxation process will, in general, be biphasic! The faster processes will occur first followed by the slower ones.<sup>1</sup>

<sup>1</sup> M. Eigen and L. De Maeyer (1974), "Theoretical Basis of Relaxation Spectrometry."

**Example**

- (1) Biphasic transient response to a jump perturbation by a system with a single intermediate, where the formation rate is slower than the dissociation rate.

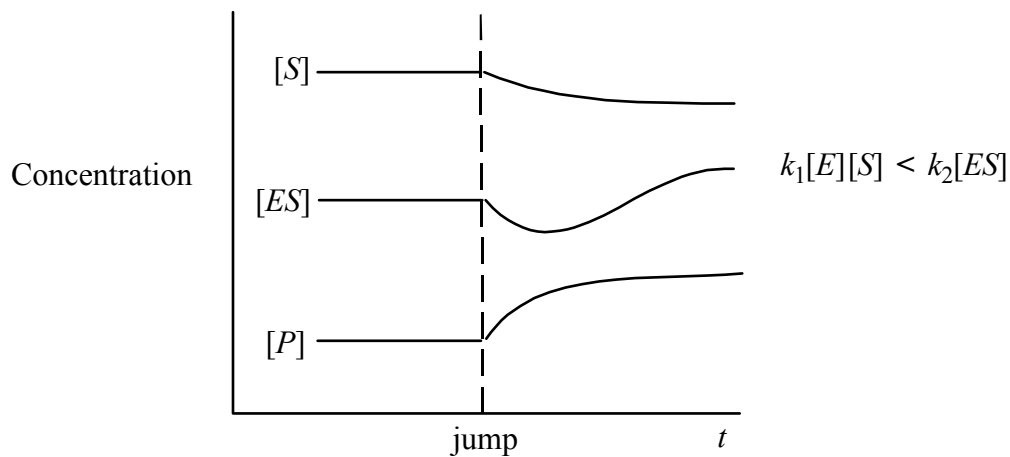


Figure 8-9

- (2) Biphasic transient response to a jump perturbation by a system with a single intermediate, where the formation rate is faster than the dissociation rate.

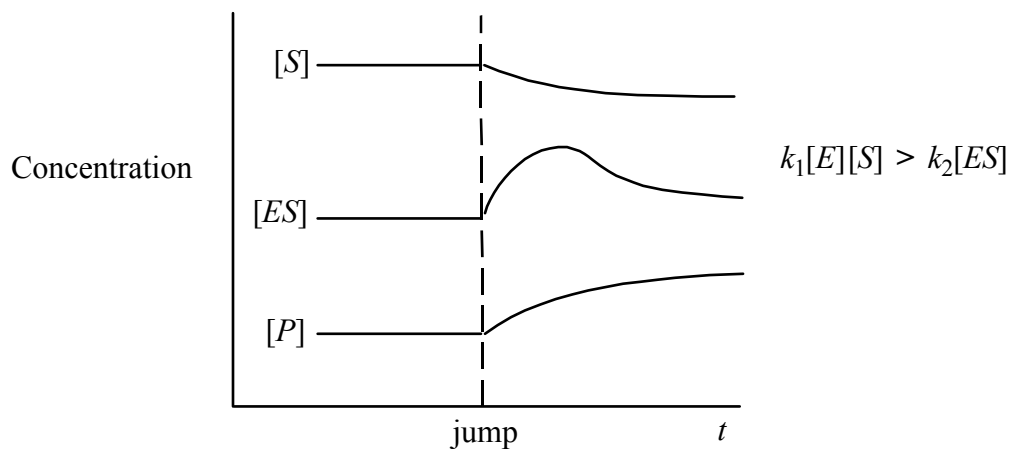


Figure 8-10